Appendix B

MOLECULAR BIOLOGY OF THE CELL

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Cell Growth and Division

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As highly organized units in a universe favoring disorder, cells are subject to wear and tear as well as to accidents. Any individual cell is therefore bound to die. If an organism is to continue to live, it must create new cells at a rate as fast as that at which its cells die. For this reason, cell division is central to the life of all organisms. In an adult human, for example, millions of cells must divide every second simply to maintain the status quo.

The process of cell division itself is strikingly visible in the microscope; it consists of two sequential processes: nuclear division (called **mitosis**) and cytoplasmic division (called **cytokinesis**). But before a typical cell can divide, it must double its mass and duplicate all of its contents. Only in this way will the two new daughter cells contain all of the components that they need to begin their own cycle of cell growth followed by division. Most of the work involved in preparing for division goes on invisibly during the growth phase of the cell cycle, which is, quite misleadingly, denoted as **interphase.**

Although a cell spends most of its lifetime in interphase and only occasional periods in the cell-division phase, most early work on the cell cycle focused on the brief division events (mitosis and cytokinesis), largely because they could be studied by direct microscopic examination. More recently, through the use of more indirect and sophisticated techniques, we have learned a considerable amount about the interphase part of the cell cycle as well. In this chapter we shall describe some of the methods currently used to study the cell cycle, consider cell-cycle regulation, and discuss several of the main events occurring during each of its different phases. Although our knowledge of the molecular basis of the cell cycle is fragmentary, wherever possible we shall try to discuss the mechanisms that are likely to be involved.

The Control of Cell Division[1,2]

Most cell components are made continuously throughout the interphase period between cell divisions. It is, therefore, difficult to define distinct stages in the progression of the growing cell through interphase. One outstanding exception is DNA synthesis, since the DNA in the cell nucleus is replicated only

Table 4–3 Landmarks in the Development of X-ray Crystallography and Its Application to Biological Molecules

1864	**Hoppe-Seyler** crystallized, and named, the protein hemoglobin.
1895	**Röntgen** observed that a new form of penetrating radiation, which he named x-rays, was produced when cathode rays (electrons) hit a metal target.
1912	**Von Laue** obtained the first x-ray diffraction patterns by passing x-rays through a crystal of zinc sulfide.
	W. L. Bragg and W. H. Bragg proposed a simple relationship between an x-ray diffraction pattern and the arrangement of atoms in a crystal that produces the pattern.
1926	**Sumner** obtained crystals of the enzyme urease from extracts of jack beans and demonstrated that proteins possess catalytic activity.
1931	**Pauling** published his first essays on "The Nature of the Chemical Bond," detailing the rules of covalent bonding.
1934	**Bernal and Crowfoot** presented the first detailed x-ray diffraction patterns of a protein obtained from crystals of the enzyme pepsin.
1935	**Patterson** developed an analytical method for determining interatomic spacings from x-ray data.
1941	**Astbury** obtained the first x-ray diffraction pattern of DNA.
1951	**Pauling and Corey** proposed the structure of a helical conformation of a chain of L-amino acids—the α-helix—and the structure of the β-sheet, both of which were later found in many proteins.
1953	**Watson and Crick** proposed the double-helix model of DNA, based on x-ray diffraction patterns obtained by **Franklin and Wilkins.**
1954	**Perutz** and colleagues developed heavy-atom methods to solve the phase problem in protein crystallography.
1960	**Kendrew** described the first detailed structure of a protein (sperm whale myoglobin) to a resolution of 0.2 nm, and **Perutz** proposed a lower-resolution structure of the larger protein hemoglobin.
1966	**Phillips** described the structure of lysozyme, the first enzyme to be analyzed in detail.
1976	**Kim and Rich** and **Klug** and colleagues described the detailed three-dimensional structure of tRNA determined by x-ray diffraction.
1977–1978	**Holmes** and **Klug** determined the structure of tobacco mosaic virus (TMV), and **Harrison** and **Rossman** determined the structure of two small spherical viruses.

Cell Culture[10]

Given the appropriate conditions, most kinds of plant and animal cells will survive, multiply, and even express differentiated properties in a tissue-culture dish. Consequently, it is possible to determine the effects on cell behavior of adding or removing specific molecules such as hormones or growth factors and to obtain homogeneous populations of cells for biochemical analysis or for studying the interactions between one cell type and another. Experiments on cultured cells are often said to be carried out *in vitro* (literally, "in glass"); the term is also used in a different sense by biochemists to refer to biochemical reactions occurring outside of living cells. While it is often a great advantage

to be able to study the complex behavior of cells in the strictly defined conditions of a culture dish, the observations must sooner or later be checked against the behavior of cells in their natural environment *in vivo*.

Cells Can Be Grown in a Culture Dish[11]

Tissue culture began in 1907 with an experiment designed to settle a controversy in neurobiology. The hypothesis under examination was known as the *neuronal doctrine*, which states that each nerve fiber is the outgrowth of a single nerve cell and not the product of the fusion of many cells. To test this contention, small pieces of spinal cord were placed on clotted plasma in a warm moist chamber and observed at regular intervals under the microscope. After a day or so, individual nerve cells could be seen extending long, thin processes into the plasma clot. Thus the neuronal doctrine was validated, and the foundations for the cell-culture revolution were laid.

The original experiments in 1907 involved the culture of small tissue fragments, or **explants.** Today, cultures are more commonly made from suspensions of cells dissociated from tissues. Such **dissociated cell cultures** (Figure 4–26) have the great advantage of enabling the experimenter to purify individual cell types from the mixture of cell types always present in a tissue and thus to examine them in isolation.

Unlike bacteria, most tissue cells are not adapted to growth in suspension and require a solid surface on which to grow and divide. This mechanical support was originally provided by the plasma clot but is now usually replaced by the surface of a plastic tissue-culture dish. Cells vary, however, in their requirements, and some will not grow or differentiate unless the culture dish is coated with extracellular matrix components, such as collagen.

Cultures prepared directly from the tissues of an organism are called **primary cultures.** In most cases, cells in primary cultures can be removed from the culture dish and used to form a large number of **secondary cultures;** they may be repeatedly subcultured in this way for weeks or months. Such cells often display the differentiated properties of the tissue from which they were obtained: fibroblasts continue to secrete collagen; cells derived from embryonic skeletal muscle fuse to form giant muscle fibers, which spontaneously contract in the culture dish; nerve cells extend axons that are electrically excitable and make synapses with other nerve cells; and epithelial cells form extensive sheets with many of the properties of an intact epithelium. Since these phenomena occur in culture, they are accessible to study in ways that are not possible in intact tissues.

Figure 4–26 Scanning electron micrograph of rat fibroblasts growing in tissue culture. (Courtesy of Gunter Albrecht-Buehler.)